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Aquatic Research and Development Section

Aquatic Research Series 2013-01

Using Environmental DNA (eDNA) as a Tool in Risk-Based Decision-Making

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January 2013

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© Queen's Printer for Ontario, 2013
Printed in Ontario, Canada

MNR 52716
ISBN 978-1-4606-1035-0 (Print)
ISBN 978-1-4606-1036-7 (PDF)

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This technical report should be cited as follows:
Wilson, C. and E. Wright. 2013. Using Environment DNA (eDNA) as a Tool in Risk-Based Decision-Making. Ontario Ministry of Natural Resources, Aquatic Research and Development Section, Aquatic Research Series 2013-01.

Cover photos clockwise from top right: collecting water sample for testing, fine-pore filter sample filtration, water samples kept cool for transport
Photo credits: D. Copplestone, A. Kidd and E. Wright, MNR

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Abstract

Environmental DNA (eDNA) is the genetic material that can be detected by sampling the non-living environment (e.g., water, soil) to provide information about what organisms may be, are, or were recently present. eDNA testing provides a unique opportunity to detect species at lower abundances than using conventional sampling approaches. A confirmed positive detection of eDNA means DNA from the species was present at that location at the time the sample was collected, but provides no information about the source of the DNA (i.e., live or dead animal, animal part, etc). Ongoing studies at MNR's Codrington Research Facility are helping to demonstrate the usefulness and limitations of the eDNA tool, and have confirmed species specificity as well as biomass and temporal sensitivity of eDNA detection. When used to provide information about invasive species and species at risk, eDNA detections can help target conventional sampling methods to increase the probability of capturing species of interest. Detections provide new information that was previously not available, helping to reduce the uncertainty associated with natural resource management decisions regarding the presence of a target species.

Résumé

L'ADN environnemental est le matériel génétique qui peut être relevé par échantillonnage du milieu non vivant (p. ex., l'eau et le sol) et qui fournit des renseignements sur les organismes qui y sont ou qui y ont été récemment présents. Les tests d'ADN constituent une meilleure façon de détecter des espèces présentes en moins grandes quantités que les méthodes d'échantillonnage traditionnelles. La détection de l'ADN environnemental indique que l'ADN d'une espèce donnée était présent au moment de la collecte de l'échantillon, mais ne fournit pas de données sur la source de l'ADN (animal mort ou vivant, partie de l'animal, etc.). Les études qui se poursuivent aux installations de recherches de Codrington du MRN servent bien à démontrer à la fois l'utilité et les limites de l'ADN environnemental et ont permis de confirmer la spécificité d'espèces ainsi que la biomasse et la sensibilité temporelle de la détection de l'ADN environnemental. Lorsqu'elles sont utilisées pour fournir des données sur les espèces envahissantes et les espèces en voie de disparition, les détections d'ADN environnemental aident à choisir les méthodes d'échantillonnage traditionnelles qui augmenteront la probabilité de capturer des espèces intéressantes. Les détections fournissent de nouvelles données, ce qui permet de réduire l'incertitude associée aux décisions en matière de gestion des ressources naturelles en ce qui a trait aux espèces cibles.

1.0 Introduction

All living organisms have genetic material (DNA[1]), that is shed into the environment from cells (skin, feces, gametes (eggs/sperm) or fluids (blood, urine, ovarian fluid), as well as from free-floating molecules from dead or degrading cells.

The presence of DNA in the environment provides information about what organisms may be, are or were recently present, when, and where.

The collection of DNA directly from the environment, termed environmental DNA or eDNA, gained wide-spread attention when used to detect Asian carp DNA in the Chicago Area Waterway System close to Lake Michigan (Jerde et al. 2011).

Using eDNA provides a unique opportunity to detect species occurrences at lower abundances than are generally detectable through conventional sampling approaches. Instead of attempting to capture the organism (species of interest) itself, environmental samples such as soil or water[2] are collected and any DNA that is present is extracted and examined using molecular genetic techniques.

What is eDNA?

Environmental DNA (eDNA) is the genetic material that can be detected by sampling the non-living environment (e.g., soil, water).

Detecting species at low abundances may be very challenging using conventional sampling methods, particularly if the initial starting place to look for these species is not known. This can result in patchy data and high uncertainty. For any species at low abundance, considerable effort is often required to determine their true presence and absence and this is particularly true for:

- rare species including species at risk,
- initial occurrences of invasive species,
- species that are difficult to capture with conventional methods, and
- species detections in habitats that are difficult to sample.

As a scientific surveillance tool, eDNA provides information about the likelihood that a species is present and can help reduce uncertainty for some resource management decisions.

A confirmed positive detection of DNA from an environmental water or soil sample means:

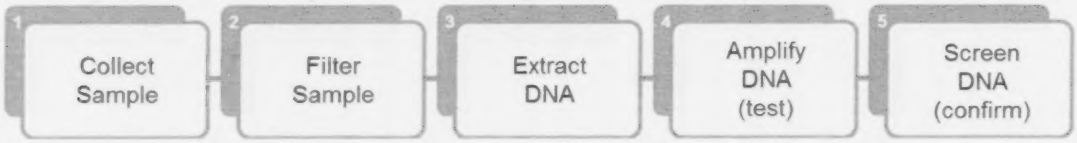
✓ DNA from the species was present at that location at the time the sample was collected, it

✗ does NOT say what the DNA source was (i.e., live animal, dead animal, animal part, etc).

[1] DNA=deoxyribonucleic acid
[2] At this time, sampling air for DNA is impractical, but with validation eDNA from airborne particles such as pollen or microorganisms may be feasible.

2.0 Method

Environmental sample collection is relatively simple and involves collecting a 1-2L sample of water or soil. The samples are filtered in the laboratory through a fine-pore filter following standard procedures developed by researchers at University of Notre Dame (Jerde et al. 2011)[3] to trap any DNA that is present.



Using PCR (polymerase chain reaction) techniques it is possible to detect and make synthetic copies of DNA present in the environmental samples collected. If no DNA is present in the environment, then no synthetic DNA copies can be made.

The PCR technique utilizes short DNA sequences (called primers). For eDNA detection, primers are designed to be species specific and bind only to DNA from the particular species of interest. The primers provide a starting point for DNA synthesis and amplification. Primers can be relatively easy to develop or acquire as new species become of interest. Environmental samples can be used repeatedly to test for more than one species of interest as new questions arise.

2.1 Quality Control

The PCR methods used are so sensitive that stringent quality controls are required at all stages (Darling and Mahon 2011):

- field sampling and lab equipment must be bleached and rinsed to destroy any traces of DNA that may cause contamination,
- a dedicated 'clean room' separate from the main lab area is needed for processing samples,
- positive and negative controls are included in all PCR analyses to prevent false results, and
- laboratory confirmation and repeated sampling is advised to verify all positive detections.

Quality assurance/quality control safeguards are essential for guarding against false positive or false negative results

[3] MNR's aquatic DNA lab is currently working with other researchers in the Great Lakes Basin to test and build upon the methods developed.

Confirmed positive: A repeatable result (amplified DNA) that is identified as the species of interest by DNA sequencing

False positive: A result in which DNA is amplified, but DNA sequencing shows the DNA is not from the species of interest

False negative: A test result that fails to amplify DNA from the species of interest when it is in fact present

2.2 Appropriate Genetic Markers

Another consideration for the application of eDNA testing is the use of appropriate genetic markers. Mitochondrial DNA (mtDNA) is the most sensitive target for species detection, as plant and animal species have hundreds to thousands of mtDNA copies per cell, versus nuclear genes which have only two copies per cell. Mitochondrial DNA is also maternally inherited without modification (no recombination), and has been extensively used for species identification and examining relationships among species.

Research completed to date by MNR has used the mitochondrial DNA cytochrome oxidase c subunit 1 (CO-1) gene as a target for species detection. The CO-1 gene is straightforward to make copies from, is effective for detecting differences between closely related species, and shows little variation within species. Importantly, this is the same genetic marker used in the international Barcode of Life (iBOL) effort led by the Biodiversity Institute, University of Guelph (Hebert et al., 2003). An existing database already contains a catalogue of CO-1 sequences for all Canadian freshwater fish species (Hubert et al., 2008) so as the global CO-1 database grows so will the ability to detect additional species from environmental samples. Current eDNA testing for aquatic species is limited by existing primers, most of which are for fish.

3.0 Limitations of the Method

Mason jar for sample collection, collected samples transported in cooler, sample filtration in lab, quantitative PCR machine for target DNA detection. Photos: A. Kidd, D. Copplestone, E. Wright, MNR

Results from current eDNA testing methods indicate only that DNA was present at the time of sampling. Therefore, although archiving samples is a potential advantage, delayed testing of

samples will describe a previous situation and mobile individuals like fish could move away from the sample site and be no longer present.

Detections of DNA indicate only that DNA was present but provides no information about the age, sex or numbers of individuals[4] that may have been present at the time of sampling. Results also do not indicate whether the DNA came from a live organism or a dead one or whether the DNA came from an intact animal/plant or a piece of one, such as a piece of skin, flower, root, or gametes (seeds, sperm, eggs). At this time, eDNA methods are not able to resolve below the species level.

Over time, DNA breaks down in the environment and detection sensitivity is limited by distance away from the original source of the DNA. Conditions that could disperse DNA include high water flow or wind, and conditions that increase the rate at which the DNA will break down include higher temperature, habitat productivity, and bacterial community. Repeat sampling is almost always required.

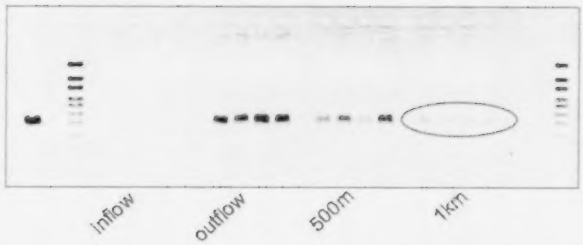
> **Storing eDNA samples:** Unfiltered water samples should be kept cold and be filtered as soon as possible. Samples may be refrigerated overnight but the DNA will degrade if kept longer. Filters with DNA can be frozen and kept until DNA is extracted.

4.0 Current MNR Research

Studies at MNR's Codrington Research Facility are measuring the effects of distance, density, and time from species arrival or removal on signal strength. The presence of multiple species at the facility (brook trout, lake trout, Atlantic salmon, and others) has also enabled tests to ensure that eDNA detection is species-specific, so that only the species of interest is detected (e.g. brook trout eDNA primers do not detect lake trout or other species).

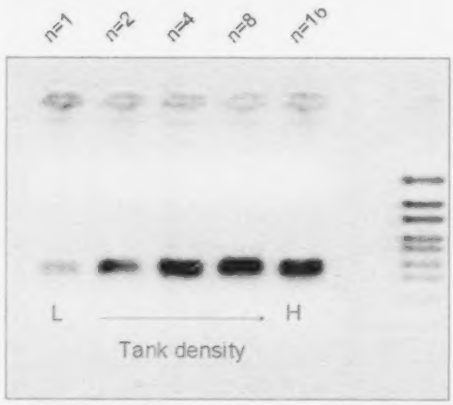
- *Distance – species detection decreases with increasing distance from the source*
    - water samples taken upstream and at increasing distances downstream of the facility showed that eDNA detection of targeted species was strongest immediately downstream of the facility, and decreased with distance (C. Wilson, unpublished data).

[4] Ongoing research using quantitative PCR (qPCR) methods may in future help to distinguish the biomass of fish present when the DNA was deposited in the environment.



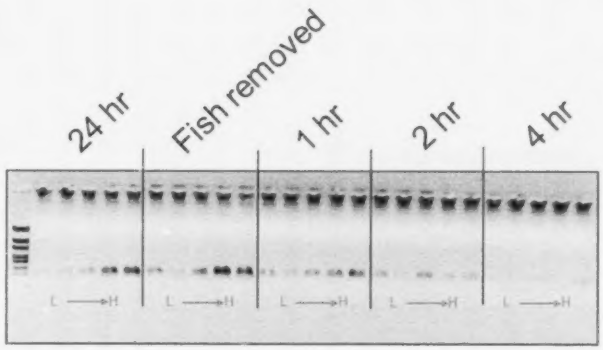
Gel image showing positive DNA detection from water samples collected at different sampling locations. Photo: J. Bronnenhuber, MNR

- *Biomass – positive DNA detection is possible at various densities (low to high)*
  - eDNA samples were collected from experimental flow-through tanks holding different densities of juvenile (young of year) brook trout (1 to 16 fish per tank). DNA was detected at all densities, and detection strength (band intensity) increased with fish densities (C. Wilson, unpublished data).



Gel image showing positive DNA detection from tanks with varying fish density. Photo: J. Bronnenhuber, MNR

- *Time – DNA signal is detectable soon after species arrival and decreases (disappears) once species is no longer present.*
  - positive detections were made from eDNA samples collected from the experimental tanks holding multiple densities of juvenile brook trout immediately after fish were added to the tanks (data not shown). Once fish were removed, detection strength decreased, and disappeared within hours of removal (C. Wilson, unpublished data).



Gel image showing positive DNA detection from tanks before and after fish removal. Photo: J. Bronnenhuber, MNR

Collaborative research with Trent University is investigating detection of aquatic species assemblages using "mini-barcode" eDNA detection (Meusnier et al., 2008) combined with next-generation sequencing (Thomsen et al. 2012). Although still in the experimental stage, this research has the potential to not only characterize local species assemblages, but also identify the presence of previously-unsuspected species.

5.0 Uses for eDNA

5.1 Rare Species Presence

Endangered, threatened or rare species tend to be difficult and labour intensive to detect using conventional and targeted sampling. A failure to detect using conventional sampling methods could occur because of low numbers of the species of interest, or their presence at a life stage that is difficult to detect with the sampling gear (i.e., too small to catch in a net or lower sensitivity to electrofishing). This could be particularly important for identifying and managing habitats occupied by species at risk, as well as monitoring changes in the dispersal of species such as unionid mussels that are dependent upon host fish species for dispersal[5].

For species at risk, the eDNA method is a sensitive tool for determining whether a failure to detect species at risk is because they are truly absent, or present and previously undetected. The eDNA method can be applied to evaluate the presence of a species, helping to determine both occurrence and distribution. The presence of a species in a particular habitat, or the seasonal use of habitat (e.g., for spawning), could advise resource management decisions by reducing uncertainty about when or where to apply habitat regulations.

Importantly, the use of environmental sampling is non-intrusive and non-disruptive because it samples the water or soil rather than the organisms themselves. It is a useful tool to augment conventional and targeted sampling efforts and can be used to focus intensive sampling in the vicinity of a positive DNA detection.

Current status of MNR testing the use of eDNA for rare species:

- eDNA sampling and analysis has been field tested for lake sturgeon, redside dace, American eel, and spotted gar;
- primers have been developed for three unionid mussels (snuffbox, mapleleaf, wavy-rayed lampmussel) and will be available once field testing is complete.

Juvenile lake sturgeon. Photo: T. Haxton, MNR

[5] Larval mussels attach to a host fish but are not detected unless host fish are examined extremely carefully. Even with careful examination there are not many fish that host larval mussels.

5.2 Invasive Species Presence

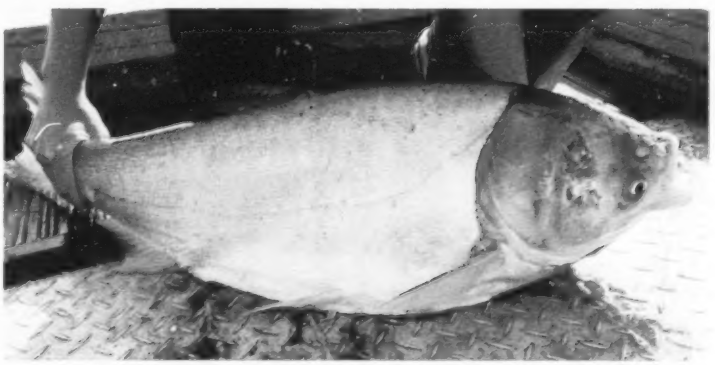
Invasive species are often well established by the time they are detected using conventional methods (i.e., nets, sightings). In general, conventional methods should not be expected to detect rare species until the individuals are abundant enough to be caught, at which time it is often too late for control or eradication efforts as the species has already become established.

Detection of an invasive species (e.g., Asian carps) using the eDNA sampling and analysis could provide an early warning and enable the launch of a monitoring response, communications, and/or management action that may help prevent the establishment or eradicate the species before complete establishment.

The eDNA method can be used at targeted locations and can inform site selection for sampling with conventional efforts. As well as providing a valuable tool for early detection of the presence of an aquatic invasive species, eDNA could be used to map introduction and expansion routes, or to test predictive models while there is still time to take preventative actions against establishment and expansion. Using eDNA as an assessment tool can increase the ability of natural resource managers to target management actions sooner and in high risk locations, enabling timely and cost-effective decisions.

Current status of MNR use of eDNA for invasive species:

- eDNA sampling and analysis has been used in a pilot surveillance program which collected water from Ontario locations along Lake Erie, Lake St. Clair, the Detroit River, and the St. Clair River. Samples were analyzed for the presence of bighead and silver carp and no DNA for either species was detected in samples collected in 2012;
- primers have been developed and validated for Northern snakehead, silver carp, bighead carp, Eurasian ruffe, rudd, round and tubenose goby, water soldier, *Hemimysis* (bloody red shrimp), *Bythotrephes* (spiny water flea), *Cercopagis* (fishhook water flea);



Bighead carp. Photo: T. Lawrence, GLFC

- candidates for future primer development include water chestnut, *Didymospenia "Didymo"* (rock snot), fanwort, and Eurasian water-milfoil.

5.3 Historical Presence

The use of eDNA may be applicable for sampling lake sediments to determine the historical presence of species in support of habitat protection or rehabilitation, and species rehabilitation and restoration efforts. As restoration decisions are often based on historical occupancy, the detection of eDNA in sediment may be the only physical evidence remaining of a species.

6.0 eDNA as a Resource Management Tool

Environmental DNA, when used as either a surveillance tool or a research tool to reduce uncertainty about the presence of a species, may help inform Ontario landscape-scale resource management decisions.

The use of eDNA surveillance data may inform resource management programs and decisions related to:

- Convention on Biological Diversity (early detection of invasive species);
- Endangered Species Act, Fish and Wildlife Conservation Act, Species at Risk Act, and the Fisheries Act;
- strategic policies such as the Ontario Invasive Species Strategic Plan and the Ontario Biodiversity Strategy;
- response plans such as the Asian Carp Response Plan for Ontario;
- recovery planning such as those for species at risk;
- regulations such as development of habitat regulations for endangered and threatened species; and
- programs such as fisheries assessment and broad-scale monitoring.

The use of eDNA sampling and analysis complements, but does not replace, conventional sampling. Detections of DNA in environmental samples indicate that there was physical evidence from the target organism present in the vicinity at the time samples were collected. For invasive species and species at risk the eDNA detections can help inform or target conventional sampling methods to increase the probability of capturing species of interest. Detections provide new information that was previously not available and helps to reduce the uncertainty regarding the presence of a target species.

The methods used for eDNA sampling and analysis continue to develop. Research is ongoing to better understand the limitations of the method as well as to determine how increased confidence in the results may be obtained.

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MNR 52716
ISBN 978-1-4606-1035-0 (Print)
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